ADAPTATION OF THE RAT TO A HIGH LACTOSE DIET. EFFECT OF THE SIZE OF THE CECUM[1]

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As a dietary constituent, lactose has some characteristics which set it apart from other common food sugars. The ingestion of fairly large quantities of lactose by man and all the laboratory species which have been tested is often followed by laxation or diarrhea, probably due to a hydragogue action of unhydrolyzed, hence unabsorbable, lactose in the intestinal tract. The feeding of equal parts of glucose and galactose is not ordinarily followed by laxation. References to the pertinent literature are found in the review by Fischer and Sutton (1949), and for the most part will not be repeated here.

In the laboratory rat fed a very high level (45–87.5%) of lactose in the diet, diarrhea, sometimes followed by death, has been reported by numerous investigators. Riggs and Beaty (1947) found that young rats exhibited transient diarrhea when fed 20, 25 or 30 percent lactose in a purified diet. Increasing the lactose level to 40 or 50 percent was followed by more severe and persistent diarrhea as well as by retarded growth. However, the rat often becomes adapted to diets containing fairly high percentages of lactose, the diarrhea subsiding after several days or weeks. In the present paper, the remission of lactose-induced diarrhea in rats maintained on a lactose diet, or failure of a test animal to develop diarrhea on a lactose diet which elicits this symptom in control rats is referred to as adaptation of the rat to lactose. Other changes in the animal, such as enlargement of the cecum, are not included in the term adaptation as used here.

In a study (Lawrence, 1953) to explore the possible mechanism of adaptation of the albino rat to a high lactose diet, evidence was obtained that an observed increase in the lactose-hydrolyzing power of the bacterial mass in the cecum might contribute to adaptation. However, the rise in the specific lactose-splitting power of the cecal flora (as judged by the specific lactase activity in the feces) was found to reach its maximum in 4 to 5 days whereas the rat did not become fully adapted to the lactose diet until several days or weeks afterwards. These facts indicate that the increased lactase specific activity of the cecal contents cannot alone be responsible for adaptation. In another study (Fischer and Sutton, 1953) it was shown that there is a moderate increase in the rate of intestinal "absorption" of lactose in rats fed 25 percent of lactose in the diet for six weeks. This increased lactose "absorption" probably is one factor in the adaptation process. Other possible factors need to be considered also. For example, several investigators have mentioned incidentally that abdominal distention and enlargement of the cecum occurs in rats fed high lactose diets. Sometimes the cecal enlargement is very great (Ershoff and Deuel, 1944). An investigation of the possible role of the enlarged cecum in adaptation seemed desirable, and is described herein.

MATERIALS AND METHODS

Albino rats from the colony of the Agricultural Biochemistry Laboratory of The Ohio State University or purchased as weanlings from the Sprague-Dawley Company were used. In any one experiment all rats were from the same source, had the same dietary history, and were approximately the same age. Equal sex distribution among groups of rats to be compared with one another was maintained.

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For feeding experiments, rats were kept in individual wire cages with coarse hardware cloth bottoms. Heavy waxed paper beneath the cages caught fecal discharges. The extent of diarrhea was estimated by counting and classifying the number of visibly separate fecal discharges collected on the paper during a 24 hr. period into 2 categories, (1) formed, and (2) soft, including the obviously fluid discharges. The percentage of soft discharges relative to the total number counted was designated as "per cent diarrhea" for that 24 hr. period.

The following diets were fed: *Corn starch diet.* This was the control diet. It contained, per kg., the following ingredients: corn starch, 670 g.; commercial casein, 240 g.; Crisco, 50 g.; U.S.P. XII salt mixture No. 2, 40 g.; choline chloride, 1 g.; inositol, 1 g.; para-animobenzoic acid, 300 mg.; calcium pantothenate, 20 mg.; nicotinamide, 15 mg.; riboflavin, 3 mg.; pyridoxine hydrochloride, 2.5 mg.; thiamine hydrochloride, 2.5 mg.; and 2-methyl-1,4-naphthoquinone, 1 mg. *Other diets.* Diets designated as lactose, cellulose and potato starch diets were prepared by replacing part of the corn starch of the control diet with the desired amount of the corresponding constituent. Cod liver oil was mixed with a small amount of corn starch diet and fed every 7 to 10 days.

To determine the dry weight of cecal tissue of sacrificed rats, gross fat was trimmed away, the colon and ileum were severed at the cecal wall and the opened cecum was washed free of debris in tap water. The tissue mass was oven dried at 105° C for 24 hr., cooled in a desiccator and weighed.

To further elucidate the importance of the cecum in adaptation of the rat to lactose, rats which weighed about 120 g. were cecectomized and an ileocolonic anastomosis was made. In general the methods for intestinal surgery given by Griffith and Farris (1942) were followed. The cecectomized animals appeared normal in 2 or 3 days, exceeded their preoperative weights in 10 days and grew normally until 3 weeks after operation when they were "challenged" with lactose.

RESULTS AND DISCUSSION

Figure 1 shows that the rat's cecum enlarged concomitantly with the feeding of a high lactose diet. Table 1 shows that this cecal enlargement in response to lactose feeding involved an increase in tissue substance (dry weight), and not merely distention of the organ.

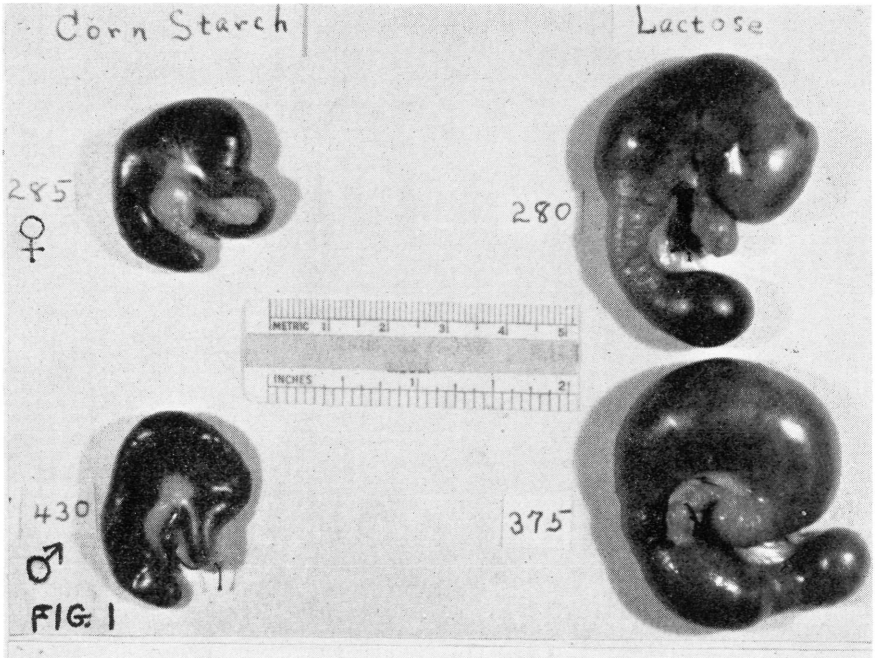
To determine the effect of a single variable, size of the cecum, upon the adaptation of the rat to a lactose diet, some way of causing cecal enlargement without feeding the animals lactose was sought. A diet containing 6 percent rice cellulose ("Ruffex") was tested, but it did not cause cecal enlargement. A diet containing 50 percent of raw potato starch was tested also, since raw potato starch has been reported to be only partly digested and to produce bulky feces in the rat and other animals (Baker *et al.*, 1949). Figure 2 shows that this diet produced cecal enlargement in the rat. Table 1 shows that this potato starch-induced enlargement involved an increase both in the fresh weight of the cecum plus its contents and in the amount (dry weight) of cecal tissue. In addition, it was observed that the fecal pellets from the rats fed the raw potato starch diet were large, soft (but well

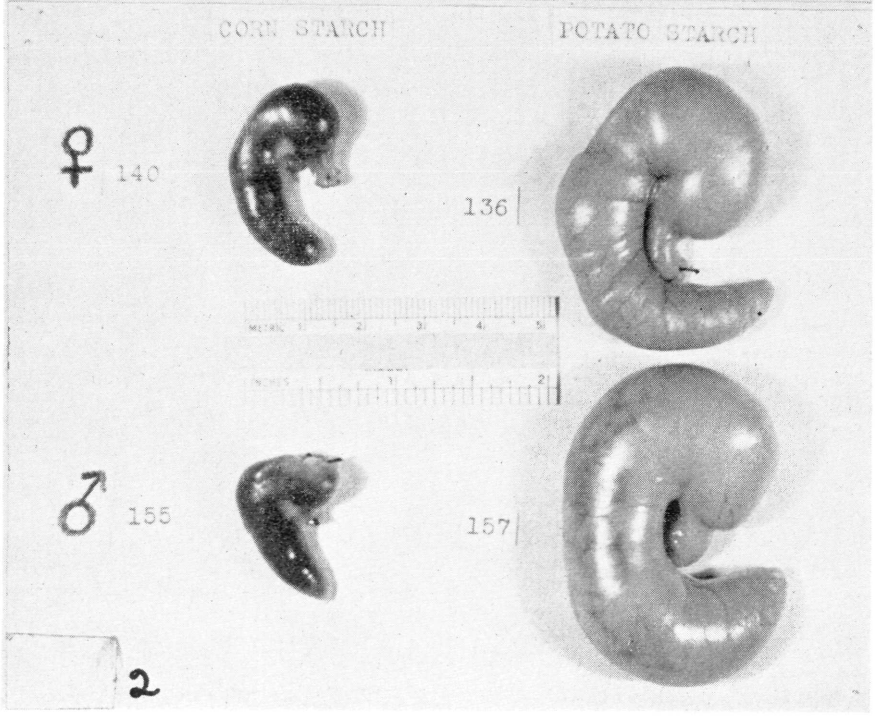
EXPLANATION OF FIGURES IN PLATE

FIGURE 1. Fresh ceca (including contents) removed from mature rats fed purified diets for 15 weeks, showing enlargement of the cecum in lactose-fed rats. The percentage of lactose in the diet was varied between 30 and 40 percent at different times.

FIGURE 2. Fresh ceca (including contents) removed from rats fed purified diets for one month following weaning, showing enlargement of the cecum in potato starch-fed rats. The potato starch diet contained 50 percent of raw potato starch.

In both figures 1 and 2, the number adjacent to each cecum indicates the body weight in grams of the rat from which the cecum was removed. The scale is indicated on the face of each figure.


Corn Starch
Lactose
285
♀
280
METRIC
INCHES
430
♂
375
FIG. 1


CORN STARCH
POTATO STARCH
♀
140
136
METRIC
INCHES
♂
155
157
2

formed) and light tan to white in color. Microscopic examination revealed many recognizable starch grains which turned blue when treated with iodine. No starch could be found in the small, black fecal pellets from the corn starch diet rats. At the end of the conditioning period, the dry weight of feces produced per rat per day was 0.22 g. and 1.75 g. in the corn starch and potato starch groups, respectively; these values represent 2.2 percent and 13.8 percent of the weight of feed consumed per day. Cecal enlargement in the rats fed the potato starch diet probably was due to this large, undigested residue which had to be handled by the alimentary system.

TABLE 1

Effect of feeding various diets upon the absolute and relative weights of the cecum

Time on diets (weeks)	Diet	No. of rats	Body weight (gm.)	Cecal Weights: Fresh, including contents (gm.)	Cecal Weights: Cleaned, dry (mg.)	Cleaned cecum dry weight: body weight ratio (mg./100 gm.)
8	Corn starch	4	267 (232–300)		135 (102–165)	52 (37–70)
8	Lactose*	4	221 (190–240)		186 (165–208)	85 (70–106)
15	Corn starch	2	358 (285–430)		155 (135–174)	44 (40–47)
15	Lactose*	2	328 (280–375)		278 (269–287)	87 (72–102)
4⅓	Corn starch	6	151 (140–190)	2.0 (1.7–2.5)	88 (62–135)	55 (36–72)
4⅓	Potato starch**	6	166 (130–215)	12.5 (9.0–15.1)	218 (160–320)	133 (90–165)

Values given are averages; ranges in parentheses. The individual data are available in Lawrence (1950).

*Percentage of lactose varied between 30 and 40 at different times.

**50 percent in diet.

After sacrificing rats from the potato starch and corn starch groups for the determination of cecal weights reported in table 1, the remaining rats were "challenged" with lactose to see if the enlarged cecum might aid the rat in adapting to a high lactose diet. The schedule of "challenging" with lactose and the results from two separate experiments are given in table 2. This table shows that rats fed lactose diets after being conditioned on the potato starch diet exhibited considerably less diarrhea than did rats fed lactose diets after being conditioned on the corn starch diet. This was true despite the fact that the potato starch-conditioned rats ate comparatively more of the lactose-containing diet, as shown by records of feed consumption.

Because adaptation of the rat to moderate percentages of lactose in the diet usually occurs in 2 to 4 weeks, the control and test groups were compared with one another for only a few days after the "challenge" feeding of the lactose diets was begun. Following the ingestion of lactose the ceca of the controls would be expected to enlarge progressively, approaching those of the test animals in size. A good deal of individual variation was encountered when the lactose level of the

TABLE 2

Diarrheal response of rats conditioned for one month after weaning on starch or potato starch diets, and subsequently fed lactose diets

Experiment	Days on lactose	Percent lactose	Percentage of diarrheal feces from rats conditioned on the diets indicated: Corn starch	Potato starch†
A*	0	0	0	0
	1	40	73	75
	2	40	94	66
	3	40	95	79
	4	25	89	60
	5	25	72	0
	6	33	86	27
	7	33	98	44
	8	33	93	57
	9	33	95	36
	Average: (Excluding day 0)		88	49
B**	0	0	0	0
	1	35	74	43
	2	35	71	46
	3	35	93	34
	4	35	91	59
	5	35	90	62
	9	35	83	37
	Average: (Excluding day 0)		84	47

*Six rats, 3 of each sex, in each dietary group.
**Twenty rats, 10 of each sex, in each dietary group.
†50 percent in conditioning diet.

TABLE 3

Effect of cecetomy on the diarrheal response of rats fed lactose

Day of experiment	Lactose fed	Percentage of diarrheal feces: Unoperated group	Cecectomized group
0	0	0	0
1	0.4 gm.*	0	34
2	0.78 gm.*	2	47
3	0	0	4
4	0	0	0
5	0	0	0
6	25**	5	78
7	25**	16	99
8	25**	38	100
9	25†	16	95
10	25†	8	95
13	25†	12	92
15	30†	6	99
21	35†	4	84
34	40†	0	89
35	40†	8	93

*Lactose fed, one dose daily, by stomach tube in 2.8 ml. water. All animals kept on corn starch diet for first five days. Feeding 3.0 ml. water by stomach tube caused no diarrhea.
**Percentage in diet of both groups.
†Percentage in diet of cecectomized group. The cecectomized group had been consuming more feed. After the eighth day the unoperated rats were fed a diet five % higher in lactose than the figures show so that they would be sure to ingest as much lactose per day as the cecectomized rats.

challenge diet was finally raised to 50 percent (data not given in table 2). Some rats, whether of the test or control group, adapted, while others (even some of the previously adapted ones) exhibited a severe, unremitting diarrhea. Thus the tolerance built up by the adapted rat may be broken down again in some individuals by raising the lactose level of the ration high enough.

In a further test of the role of the cecum in the adaptation process, the cecum was removed from 7 young rats (5 males and 2 females). Three weeks later these cecectomized rats and seven unoperated controls, all of which had been fed the corn starch diet since weaning, were "challenged" with lactose according to the schedule in table 3. This table shows that the cecectomized rats, when fed lactose by stomach tube or in the diet, passed very high percentages of diarrheal feces compared to the unoperated control rats similarly fed lactose. The table shows also that, on the average, the cecectomized group failed to adapt to lactose in the diet. However, one rat in the cecectomized group did adapt almost completely by the twenty-first day. At necropsy the colon of this rat was found to be fairly large and well-filled with a soft, black fecal mass throughout its length. The colons of most of the other cecectomized rats were almost empty. Possibly hypertrophy of the colon eventually could enable that organ to take over much of the function of the missing cecum.

SUMMARY

1. Albino rats were fed a purified diet containing corn starch as the basal carbohydrate, and other rats were fed the same diet but with 30 to 40 percent of lactose or 50 percent of raw potato starch substituted for part of the corn starch. Compared to the cecum in the corn starch-fed control rats, the cecum in the rats fed the lactose or potato starch diets was enlarged. This enlargement involved not merely distention of the organ by a large cecal mass but also an increase in the dry weight of the cecal tissue.

2. When rats were conditioned for one month on the potato starch or the corn starch diets and subsequently were fed a diet high in lactose, the potato starch-conditioned (large cecum) animals exhibited considerably less diarrhea than the corn starch-conditioned (small cecum) controls.

3. Only one out of seven cecectomized rats adapted (*i.e.*, recovered from the initial diarrhea) during a 35-day feeding period on a dietary regimen involving increasing percentages of lactose. Unoperated control rats adapted almost completely during this period.

4. The size of the cecum appears to be a significant factor in the adaptation of the rat to a high lactose diet.

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